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Original article

Characterization of *Listeria monocytogenes* isolated from human clinical cases

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ABSTRACT

Introduction: *Listeria monocytogenes* is an emerging foodborne pathogen and causative agent of listeriosis. It is one of the cause of spontaneous abortions and meningitis. The objective of the study was to determine the incidences of *L. monocytogenes* from human cases of spontaneous abortions or having a history of spontaneous abortions and meningitis. **Materials and Methods:** A total of 481 samples from humans having a history of spontaneous abortions and meningitis were analyzed. Isolation of the pathogen was attempted employing two step enrichment followed by plating on selective media. Characterization of the isolates was based on biochemical tests, haemolysis on blood agar, CAMP test, phosphatidyl inositol-specific phospholipase C assay, multiplex PCR to detect virulence-associated genes *(hlyA, actA* and *iap*) and serotyping by conventional and PCR based method. **Results:** Of the 481 samples analysed, 7 (1.45%) samples from abortion cases were found positive, while no CSF sample from meningitis cases was positive for *L. monocytogenes*. All the isolates showed in-vitro virulence characteristics such as hemolysis on 5% sheep blood agar, positive CAMP test and showed hallow formation on ALOA medium. All the isolates showed the presence of the *hly*, *act*A and *iap* genes. Conventional serotyping as well as PCR based serotyping revealed the isolates to be of 4b, 1/2b and 4e serotypes. Antibiotic sensitivity assay showed that the isolates were sensitive to trimethoprim, however, the isolates were least sensitive to meropenem. **Conclusions:** The study highlights the incidence of *L. monocytogenes* in humans with spontaneous abortions.

KEYWORDS: *Listeria monocytogenes,* Serotypes, Isolation, PCR, human.

INTRODUCTION

Listeriosis is a foodborne illness of major public health concern because of the severity of the disease and its high case fatality rate. The genus *Listeria* consists of 10 species namely, *L. monocytogenes, L. ivanovii, L. seeligeri, L. innocua, L. grayi, L. welshimeri, L. rocourtiae, L. marthi, L. weihenstephanensis* and *L. fleischmannii.* Recently, five new species of *Listeria, L. floridensis, L. aquatica, L. cornellensis, L. riparia,* and *L. grandensi* have been decribed [1] Amongst these species *L. monocytogenes* and *L. ivanovii* are the known pathogenic species of which *L. monocytogenes* is a human pathogen and *L. ivanovii* is an animal pathogen which on rare occasions can cause infection in humans [2]. The manifestations of listeriosis in humans are in the form of meningitis, septicemia, abortions, gastroenteritis, endocarditis, perinatal and neonatal septicaemia.

Most of the patients suffering from listeriosis have an underlying condition which leads to impairment of T-cell mediated immunity [3]. A yearly burden of listeriosis per year has been estimated as 23,150 illnesses, 5463 deaths, and 1,72,823 disability-adjusted life years (DALYS) [4]. In India this pathogen has been listed in Food Safety and Standards Act 2006.

Due to rapid urbanization and globalization, climatic changes, changes in microbial and other ecological systems, decreasing freshwater supplies, degradation of sanitation and the immediate human environment, it is expected that the trend of infectious diseases will increase [5]. Consequently, it can be expected that food borne diseases will also increase, especially in the developing countries.

Reliable diagnosis of listeriosis is made by cultural methods, however, the isolation as well as the differentiation of pathogenic and non-pathogenic *Listeria* remains a time consuming and tedious task [6,7]. Serotyping has been widely used to characterize *L. monocytogenes* epidemiologically [8,9]. Although 12 serotypes of *L. monocytogenes* have been reported, only three serotypes (1/2a, 1/2b and 4b) are frequently isolated from the clinical cases and human epidemics [10]. As most of the strains isolated from human clinical, food and environment belong to a small number of serotypes, highly discriminatory typing methods that correlate with serotyping are necessary [11,12]. *Listeria* has been isolated from a variety of foods in India such as milk and milk products, meats of goat and sheep, raw fish and processed fish and meat products and human clinical cases [13, 14].

Though the listeriosis is a rare infection, it is about 20 times more common in pregnant women than in the general population [15]. Pregnant women account for 27% of all listerial infections, which can cause mild illness in mothers, but can be devastating to the fetus, in some cases leading to severe disease or fetal death [15]. Though the listeriosis in pregnancies and meningitis cases have been reported worldwide, very few reports are available in case of India. However considering the environmental conditions, traditional food habits and increased use of refrigerated ready-to-eat foods the incidences of listeriosis cannot be denied. The objective of the investigation was to study the incidence of *Listeria* in spontaneous abortions and meningitis cases.

MATERIALS AND METHODS

Bacteria

The strains of *L. monocytogenes* 4b (MTCC 1143), *Staphylococcus aureus* (MTCC 1144) and *Rhodococcus equi* (MTCC 1135) used in the study were obtained from the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

Samples

A total of 481 clinical samples comprised of urine (318), faeces (22), placental bits (119), vaginal and cervical swabs (12) from cases of spontaneous abortions or having a history of spontaneous abortions and cerebrospinal fluid (10) from cases of meningitis were collected from private and government hospitals in Goa and of Maharashtra during the period from September 2009 to June 2013. Samples were collected in sterile containers and transported on ice to the laboratory for analysis. This study was approved by the institutional review board of the hospitals and was deemed exempt from informed consent.

Isolation of Listeria

Isolation of listeriae from the faecal, vaginal, urine samples and placental bits was attempted as per the US Department of Agriculture (USDA) method described by McClain and Lee [16] after making necessary modifications. Briefly, approx. 5 ml of each of the urine samples and 25 g of the placental tissue were inoculated into 45 ml and 225 ml of University of Vermont Medium (UVM) I, respectively and incubated for 24 h at 37^{o}C. 0.1 ml of UVM I was then inoculated into UVM II and incubated for 24 h at 37^{o}C. A loopful from UVM II was then streaked on Polymyxin, acriflavine, lithium chloride, ceftazidime, aesculin, mannitol (PALCAM) agar. The agar plates were incubated at 37^{o}C for 48 h.

Each of the collected swabs (faecal and vaginal) was aseptically inoculated into 10 ml of UVM-1 and incubated overnight at 37^{o}C. The enriched UVM-1 inoculum (0.1 ml) was then transferred to UVM-2 broth and again incubated overnight at 37^{o}C. The inoculum enriched in UVM-2 was streaked directly on PALCAM agar and the plates were incubated at 37^{o}C for 48 h. The grey green colonies with black sunken centres from PALCAM plates were suspected to be listeriae. The presumed colonies of *Listeria* (at least three per plate) were further confirmed.

Identification of Listeria

Isolated typical colonies were then picked up and inoculated into Brain Heart Infusion Broth (BHI) for further characterization. All the isolates were subjected to biochemical tests such as catalase, oxidase, nitrate reduction, methyl red and Voges Proskaur tests. Sugar fermentation tests by using mannitol, xylose, rhamnose and α-methyl D-mannoside were carried out. The isolates were identified based on the criteria suggested by Lovett [17, 18]. Motility test was carried out at 25^{o}C. All the biochemically characterized *Listeria* isolates were further subjected to haemolysis on sheep blood agar (SBA); Christie, Atkins, Munch Petersen (CAMP) test and detection of phosphatidylinositol-specific phospholipase C (PI-PLC) activity on agar *Listeria* according to Ottaviani and Agosti (ALOA) medium (Himedia Labs, Mumbai, India), multiplex PCR targeting virulence-associated genes (*act*A, *hly*A and *iap*), conventional serotyping and PCR based serotyping [8].

PCR

The primers for detection of hemolysin gene (*hly*A), actin gene (*act*A) and p60 gene (*iap*) of *L. monocytogenes* used in this study were synthesized by Sigma-Aldrich, USA. The details of the primer sequences are shown in Table 1. The genomic DNA of all the isolates was extracted using bacterial DNA extraction kit (Chromous Biotech, Bangalore, India) and detection of virulence genes was carried out by a multiplex PCR method to identify presence of the *hly*, *act*A and *iap* genes in the isolates [6]. Reaction was set for 25µl reaction volume and performed by Master Cycler epGradient (Eppendorf, Germany) with a preheated lid. The completed reaction mixture was subjected to an initial denaturation at 95^{0}C for 2 min followed by 35 cycles each of 15 s denaturation at 95^{0}C, 30 s annealing at 60^{0}C and 1 min 30 s extension at 72^{0}C. It was followed by final extension of 10 min at 72^{0}C and held at 4^{0}C.

The primers used for multiplex-PCR serotyping were synthesized from Sigma Aldrich, USA. The details of

primers used in the study are given in Table 1. The multiplex-PCR serotyping was standardized as described by Doumith et al. [8]. Fifty microliter reaction mixtures were prepared each containing 2 U Taq DNA Polymerase, 10x PCR Buffer (50 mM Tris–HCl, 10 mM KCl, 50 mM Ammonium Sulphate, 2 mM MgCl2), 300 µM dNTP mix, 2 mM MgCl2, 2 µM each of primer *lmo0737*, *ORF2819*, *ORF2110* and *prs* and 10 µg/ml of DNA template. PCR was performed in Master Cycler epGradient Thermocycler (Eppendorf, Germany) having a pre-heated lid with an initial denaturation step at 94ºC for 5 min, 35 cycles of 94ºC for 30 s, 54ºC for 1 min 15 s, and 72ºC for 1 min 15 s, and final extension at 72ºC for 10 min. Samples were held at 4ºC until electrophoresis. Eight microliter of PCR product was separated by electrophoresis in 1.5% agarose gel stained by ethidium bromide.

Conventional serotyping

The isolates were serotyped using commercial *Listeria* antisera (Denka Seiken, Japan) in accordance with the manufacturer's instructions.

Antibiotic sensitivity testing

Antibiotic susceptibility pattern of all the isolates were determined by the standard disc diffusion method [19]. Antibiotic discs used were ampicillin (25g/disc), gentamicin (10µg/disc), kanamycin (5µg/disc), chloramphenicol (30µg/disc), ciprofloxacin (30µg/disc), erythromycin (5µg/disc), meropenem (10µg/disc), trimethoprim (25µg/disc), vancomycin (30µg/disc) and penicillin(10µg/disc). The results were interpreted as per the NCCLS criteria [20].

Table 1 : Details of primers used in present study for amplification of virulence and serotype marker in *Listeria* spp.

Target Gene	Primer sequence	Product size (bp)	Reference
*hly*A	Forward 5'-GCAGTTGCAAGCGCTTGGAGTGAA-3'	456	[39]
	Reverse 5'- GCAACGTATCCTCCAGAGTGATCG-3'		
iap	Forward 5'-ACAAGCTGCACCTGTTGCAG-3'	131	[33]
	Reverse 5'-TGACAGCGTGTGTAGTAGCA-3'		
*act*A	Forward 5'-CGCCGCGGAAATTAAAAAAAGA-3'	839	[40]
	Reverse 5'- ACGAAGGAACCGGGCTGCTAG-3'		
lmo0737	Forward AGGGCTTCAAGGACTTACCC	619	[8]
	Reverse ACGATTTCTGCTTGCCATTC		
lmo1118	Forward AGGGGTCTTAAATCCTGGAA	906	[8]
	Reverse CGGCTTGTTCGGCATACTTA		
ORF2110	Forward AGTGGACAATTGATTGGTGAA	597	[8]
	Reverse CATCCATCCCTTACTTTGGAC		
ORF2819	Forward AGCAAAATGCCAAAACTCGT	471	[8]
	Reverse CATCACTAAAGCCTCCCATTG		
prs	Forward GCTGAAGAGATTGCGAAAGAAG	370	[8]
	Reverse CAAAGAAACCTTGGATTTGCGG		

RESULTS

The microbiological analysis of 481 samples revealed 7(1.45%) samples to be positive for *L. monocytogenes*. The seven isolates from clinical samples were recovered from one placental bit sample, two urine samples, one vaginal swab and three from faecal samples. No isolates were obtained from CSF. All the isolates were haemolytic, CAMP positive with *Staphylococcus aureus*, showed greenish colored zones along with a translucent halo around the colonies of the isolates on ALOA. Multiplex PCR for the virulence genes showed the presence of *hly*, *act*A and *iap* genes in all the isolates (Table 2).

The isolates were subjected to conventional serotyping and multiplex PCR based serotyping. The isolates were serotyped as 1/2b (1), 4b (4) and 4e (2) (Table 2, Fig. 1).

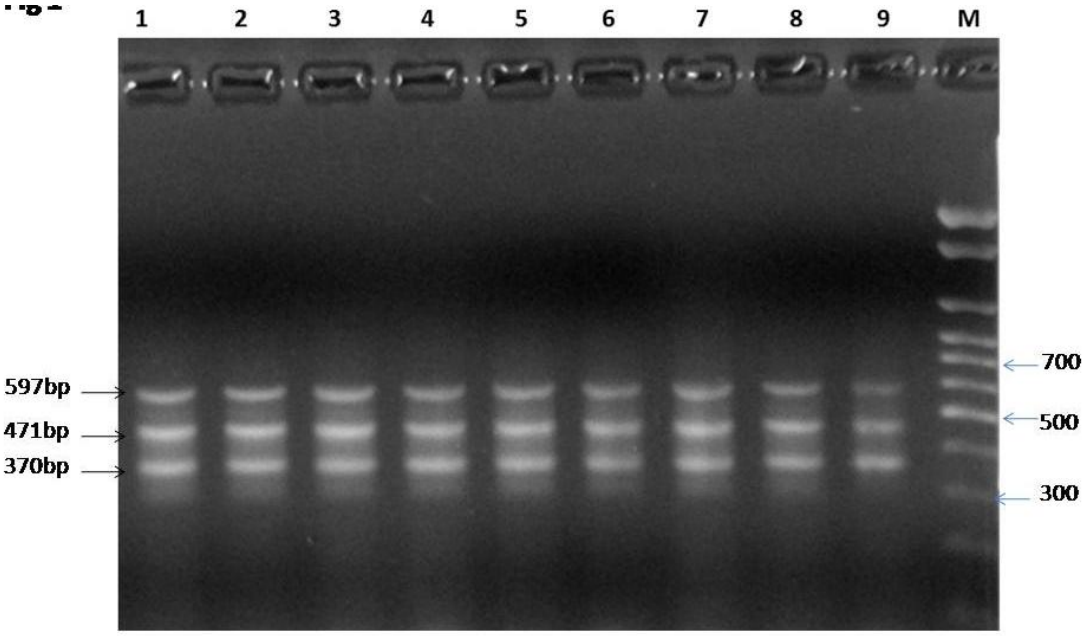
All the isolates of *L. monocytogenes* showed highest sensitivity (100%) against trimethoprim followed by chloramphenicol and ciprofloxacin (90%), and gentamicin (80%). The isolates were highly resistant against erythromycin and meropenem and least sensitive against vancomycin, penicillin (10%) and ampicillin and kanamycin (20%).

Table 2 : Characterization of *Listeria monocytogenes* isolated from human cases.

Isolate	CAMP with Haemolysis		PI-PLC	Virulence genes		Serotype	
	With S/R	on SBA	Assay	*hly* A	*act* A	*iap*	
KI3	+S	++	+	+	+	+	½ b
KM9	+S	++	+	+	+	+	4b
GMI23	+S	++	+	+	+	+	4e
GMII 292	+S	++	+	+	+	+	4e
2 Sush	+S	++	+	+	+	+	4b
A	+S	++	+	+	+	+	4b
D	+S	++	+	+	+	+	4b

CAMP: Christie, Atkins, Munch-Petersen test. +S: Enhanced zone of haemolysis with *Staphylococcus aureus*.
PI-PLC : Phosphotidylinositol-specific phospholipase C, SBA : Sheep blood agar

Fig. 1. Multiplex PCR based serotyping for determination of the serogroups of the isolates *Listeria monocytogenes.*



Lanes 1-8: *L. monocytogenes* serogroup 4b, 4d, 4e; lane 9: *L. monocytogenes* serotype 4b MTCC 1143, lane M: 1 Kb DNA ladder

DISCUSSION

Listeria monocytogenes is a ubiquitous bacterium found in soil, decaying vegetation and the faeces of animals. Infection of *L. monocytogenes* causes listeriosis among the immuno-compromised, pregnant women, the unborn, newborns and the elderly. Industrially processed food is been frequently linked as source of infection. Several studies shows that, *L. monocytogenes* enters into food chain easily and depending upon the innate capability of the strain and environmental conditions get colonies into food processing environment. Considering the listeriosis worldwide outbreak scenario and comparative lifestyle and food habits in India, the probability of such outbreaks cannot be denied. In developed countries, due to its clinical severity and high fatality rate, invasive listeriosis is of great concern to public health despite its low incidence [18].

Significant increasing trends in listeriosis notification rates were noted in Austria, Denmark, Hungary, Italy, Spain and Sweden from 2005 to 2009 [21,22]. However, there is lack of knowledge of listeriosis both of sporadic as well as of epidemic nature in India.

Among the different forms of listeriosis, abortion and meningitis are the most common forms observed. In the present study, *L. monocytogenes* was isolated from 1.45% samples collected from humans with spontaneous abortions. The isolates were recovered from placental bit, urine samples, vaginal swab and from faecal samples. In earlier studies from India, Dhanashree et al. [13] isolated *L. monocytogenes* from 3.1% placental bit samples. Kaur et al. [14] reported *L. monocytogenes* from 3.2% placental bits and 1.6% vaginal swabs. In a recent study, *L.*

monocytogenes was isolated from 5.3 % of placental bits and 1.3% of vaginal swabs [23]. Abdel Malek et al. [24] reported an isolation rate of 7.14% for *Listeria* spp. from faecal samples of children in a paediatric hospital in Egypt. Gupta et al. [25] reported the isolation of *L. monocytogenes* from blood samples. Bhujwal et al. [26] could not isolate *Listeria* from CSF samples. Rate of isolation of *L. monocytogenes* was low from urine samples. Following delivery, *L. monocytogenes* may be shed in vaginal secretions or urine of mothers of infected newborns for 7–10 days [27]. The pathogen has also been reported from urine of a subject with repeated abortions [28].

The majority of isolates observed in this study to the 4b serotype which has been implicated in causing epidemics of human listeriosis [8,9,29]. Serotype 1/2b has been implicated in sporadic cases of listeriosis. In Sweden, 18% of the human isolates in 2010 were reported to be of serotype 4b [30]. In another study, 154 isolates of *L. monocytogenes* isolated in Spain were grouped into four serotypes, 4b (61%), 1/2b (19%), 1/2a (18%) and 1/2c (2%), with 100% of susceptibility to ampicillin and cotrimoxazole [31]. In a study in Greece, serotype group 4b, 4d, 4e was detected in 39.4%, among both clinical and food isolates while only serotype group 1/2a, 3a (23.0%) was detected from among food isolates [32]. All three isolates of *L. monocytogenes* from animal samples belonged to serotype 4b. The high incidence of the 4b serotype indicated the threat of epidemics if adequate preventive measures are not employed.

All seven isolates of *L. monocytogenes* were examined for the presence of virulence genes. All the isolates irrespective of their source showed the presence of the *act*A, *hly*A and *iap* genes. Multiple virulence factors such as hemolysin (*hly* A), actin polymerization protein (*act* A) and invasive associated protein (*iap*) are important in pathogenesis of *L. monocytogenes* [33,34]. The presence virulence associated genes indicated the pathogenic nature of the isolates.

With the time, bacteria have evolved to tackle the antibiotics forming resistance. Therefore, it demands the consistent monitoring of antibiotic profile of a bacterium, particularly pathogens. As *L. monocytogenes* strains from this study are pathogenic and of clinical interest, sensitivity to the antibiotics that are currently in practice for listeriosis was determined. It was interesting to note the least sensitivity against ampicillin, penicillin, vancomycin and kanamycin.

β-lactam antibiotics such as ampicillin and penicillin are drug of choice [35], however, the isolate seems to be resistant to ampicillin and penicillin. Though, the resistance to *L. monocytogenes* has been documented in 1989 in France [36], several researchers have noted the resistance to penicillin, trimethoprim, tetracycline, erythromycin, nalidixic acid etc. [23]. In India, antibiotic resistance strains of *L. monocytogenes* from food and clinical sources were reported sporadically [5,23,37]. Nigam et al. [38] reported that isolates of *Listeria spp.* were 100% sensitive to ampicillin, chloramphenicol, penicillin and 88% sensitive to ciprofloxacin and gentamicin. Increase in resistance to antibiotics like erythromycin, ampicillin and penicillin which are widely used in the treatment could pose a problem in treatment of listeriosis.

CONCLUSION

The study shows incidence of listeriosis among the spontaneous abortion cases. This could be the result of decreased sensitivity of the *L. monocytogenes* toward traditional antibiotics. Pregnant women should be educated for listeriosis and should avoid ready-to-eat foods that are stored for long time refrigeration, raw milk or related products, meat products etc. Proper protein rich diet to keep the immune system strong must be maintained. Repeated abortion cases should be analyzed for *L. monocytogenes*. In case of proved listeriosis cases, combined antibiotic therapy should be used rather than a single antibiotic to avoid the resistance problem.

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REFERENCES

1. den Bakker HC, Warchocki S, Wright EM, Allred AF, Ahlstrom C, Manuel CS, et al. *Listeria floridensis* sp. nov., *Listeria aquatica* sp. nov., *Listeria cornellensis* sp. nov., *Listeria riparia* sp. nov. and *Listeria grandensis* sp. nov., from agricultural and natural environments. Int J Syst Evol Microbiol. 2014 :1882-1889.
2. Guillet C, Join-Lamber O, Le Monnier A, Leclercq A, Mechaï F, Mamzer-Bruneel MF, et al. Human listeriosis caused by *Listeria ivanovii*. Emerg Infect Dis 2010;16:136-138
3. Allerberger F, Wagner M. Listeriosis: a resurgent foodborne infection. Clin Microbiol Infect 2010;16:16-23.
4. de Noordhout CM, Devleesschauwer B, Angulo FJ, Verbeke G, Haagsma J, Kirk M, et al. The global burden of listeriosis: a systematic review and meta-analysis. Lancet Infect Dis 2014; 14:1073-1082.
5. Tirumalai P. Listeriosis and *Listeria monocytogenes* in India. Wudpecker J Food Technol 2013;16:98-103.
6. Rawool DB, Malik SV, Shakuntala I, Sahare AM, Barbuddhe SB. Detection of multiple virulence associated genes in *Listeria monocytogenes* from bovine mastitis cases. Int J Food Microbiol 2007;113:201-207.
7. Jadhav S, Bhave M, Palombo EA. Methods used for the detection and subtyping of *Listeria monocytogenes*. J Microbiol Methods 2012;88:327-341.
8. Doumith M, Buchriester C, Glaser P, Jacquet C, Martin P. Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. J Clin Microbiol 2004;42:3819–3822.
9. Liu D. Identification, subtyping and virulence determination of *Listeria monocytogenes*, an important

foodborne pathogen. J Med Microbiol 2006;55:645–659.
10. Chen J, Chen Q, Jiang J, Hu H, Ye J, Fang W. Serovar 4b complex predominates among *Listeria monocytogenes* isolates from imported aquatic products in China. Foodborne Patho Dis 2010;7:31–41.
11. Orsi RH, den Bakker HC, Wiedmann M. *Listeria monocytogenes* lineages: Genomics, evolution, ecology, and phenotypic characteristics. Int J Med Microbiol 2011;301:79–96.
12. Barbuddhe SB, Malik SVS, Kumar AJ, Kalorey DR, Chakraborty T. Epidemiology and management of listeriosis in India. Int J Food Microbiol 2012;154:113-118.
13. Dhanashree B, Otta SK, Karunasagar I, Goebel W. Karunasagar I. Incidence of *Listeria* spp. in clinical and food samples in Mangalore, India. Food Microbiol 2003;20:447-453.
14. Kaur S, Malik SVS, Vaidya VM, Barbuddhe SB. *Listeria monocytogenes* in spontaneous abortions in humans and its detection by multiplex PCR. J Appl Microbiol 2007;103:1889–1896.
15. Janakiraman V. Listeriosis in pregnancy: Diagnosis, treatment and prevention. Rev Obst Gynecol 2008;1:179-1785.
16. McClain D, Lee WH. Development of USDA-FSIS method for isolation of *Listeria monocytogenes* from raw meat and poultry. J Asso Off Anal Chem 1988;71:660–664.
17. Lovett J. Isolation and identification of *Listeria monocytogenes* in dairy products. J Asso Off Anal Chem 1988;71:658-660.
18. Jones D, Seeliger HPR. The genus *Listeria*. In: Balows A, Trüper H, Dworkin M, Harder M, Schleifer KH. editors. The Prokaryotes: A Handbook on the biology of bacteria: ecophysiology, isolation, identification, applications, 2nd edition. Springer-Verlag, New York; 1992. pp. 1595–1616.
19. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Amer. J Clin Pathol 1966;45:493–496.
20. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests. Approved standard. NC-CLS document M02-A11. National Committee for clinical Laboratory standards, Wyane, PA, 2012. http://antimicrobianos.com.ar/ATB/wp-content/uploads/2012/11/01-CLSI-M02-A11-2012.pdf
21. Pontello M, Guaita A, Sala G, Cipolla M, Gattuso A, Sonnessa M, et al. *Listeria monocytogenes* serotypes in human infections (Italy, 2000-2010). Ann Istit Super Sanità 2012;8:146-150
22. Parihar VS, Lopez-Valladares G, Danielsson-Tham ML, Peiris I, Helmersson S, Unemo M, et al. Characterization of human isolates of *Listeria* monocytogenes in Sweden 1986-2007. Foodborne Path Dis 2008;5:755-761.
23. Soni D, Singh R, Singh D, Dubey S. Characterization of *Listeria monocytogenes* isolated from Ganges water, human clinical and milk samples at Varanasi, India. Infect Gen Evol 2013;14: 83-91.
24. Abdel Malek A, Hassan AS, Hassanein R, Mohamed MA, Elsayh KI. Occurrence of *Listeria* species in meat, chicken products and human stools in Assiut city, Egypt with PCR use for rapid identification of *Listeria monocytogenes*. Vet World 2010;3:353-359.
25. Gupta R, Ramachandran VG, Gupta P. Listeriosis: an opportunistic infection. Indian J Pediat 1997;34:732–734.
26. Bhujwala RA, Chandra RK, Hingorani V. *Listeria* meningitis in Delhi (A pilot study). Ind J Med Res 1974;62:1333-1336.
27. Anon. Reporting and Surveillance guidelines for listeriosis. Communicable Disease Epidemiology, Office of Epidemiology, Washington State Department of Health, Shoreline, USA, 2002. Available at: http://www.doh.wa.gov/Notify/guidelines/listeriosis.htm. (Assessed on 14th January, 2014).
28. Nicolosi VM, Gismondo MR, Russo G, Toscano MA. *Listeria monocytogenes* isolation from a urine specimen of a subject with repeated abortions. Ann Sclavo 1978;20:692–695.
29. Farber JM, Peterkin PI. *Listeria monocytogenes*, a food-borne pathogen. Microbiol Rev 1991;55, 476-511.
30. Lambertz ST, Ivarsson S, Lopez-Valladares G, Sidstedt M, Lindqvist R. Subtyping of *Listeria monocytogenes* isolates recovered from retail ready-to-eat foods, processing plants and listeriosis patients in Sweden 2010. Int J Food Microbiol 2013;166:186–192.
31. Lepe JA, Torres MJ, Liró J, Luque R, Aznar J, Grupo LISAND-Microbiología. Microbiological characterisation of *Listeria monocytogenes* isolates from human cases in Andalusia. Enferm Infecc Microbiol Clin 2012; 30:602-607.
32. Houhoula DP, Peirasmaki D, Konteles SJ, Kizis D, Koussissis S, Bratacos M, et al. High level of heterogeneity among *Listeria* monocytogenes isolates from clinical and food origin specimens in Greece. Foodborne Pathog Dis 2012; 9:848-852
33. Furrer B, Candrian U, Hoeflein C, Luethy J. Detection and identification of *Listeria monocytogenes* in cooked sausage products and in milk by in vitro amplification of haemolysin gene fragments. J Appl Bacteriol 1991;70:372-379.
34. Portnoy DA, Chakraborty T, Goebel W, Cossart P. Molecular determinants of *Listeria monocytogenes* pathogenesis. Infect Immun 1992; 60:1263-1267.
35. Temple ME, Nahata MC. Treatment of listeriosis. Ann Pharm 2000;34:656-661.

36. Poyart-Salmeron C, Carlier C, Trieu-Cuot P, Courtieu AL, Courvalin P. Transferable plasmid-mediated antibiotic resistance in *Listeria monocytogenes*. Lancet 1990; 255:1422–1426.
37. Gupta S, Sharma V. Antibiotic resistance pattern among different *Listeria* species isolated from mutton and chevon. J Ani Res 2013;3:99-102.
38. Nigam, P, Katoch RC, Verma S, Batta MK. Chemotherapeutic sensitivity profile of *Listeria* species from reproductive disorders of domestic animals. Indian Vet J 1998; 75:658-659.
39. Paziak-Domanska B, Bogusławska E, Wieckowska-Szakiel M, Kotłowski R, Rózalska B, Chmiela M, et al. Evaluation of the API test, phosphatidylinositol-specific phospholipase C activity and PCR method in identification of *Listeria monocytogenes* in meat foods. FEMS Microbial Lett 1999;171:209–214
40. Suárez M, González-Zorn B, Vega Y, Chico-Calero I, Vázquez-Boland JA. A role for ActA in epithelial cell invasion by *Listeria monocytogenes*. Cell Microbiol 2001;3:853–864.

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